STUDIES ON THE NECESSITY OF THE BRAIN FOR THE INCEPTION OF INSECT METAMORPHOSIS.

STEFAN KOPEĆ,

GOVERNMENT INSTITUTE FOR AGRICULTURAL RESEARCH, PULAWY, POLAND.[1]

WITH 2 TABLES AND 4 FIGURES IN TEXT.

CONTENTS.

Gudernatsch ('12, '14) was the first to describe the acceleration of metamorphosis in tadpoles fed on thyroid. These results were confirmed for different amphibians by Laufberger ('13), Cotronei ('14), Brendgen ('14), Abderhalden ('15, '19), Kaufman ('17, '18), Kahn ('16), Hirschler ('18–'19), etc., and first of all by Romeis ('14–'19). Adler ('16) ascertained that changes of temperature of the environment cause changes of celerity of metamorphosis in tadpoles, together with simultaneous differences in the structure of the thyroid.

The results of the investigations of Loeb ('96) and Wintrebert ('05–'11), who cut the spinal cord or the nerves of larvæ, contradict the influence of the brain on metamorphosis of amphibians, or at least the influence by means of nerves. On the other hand, Babák ('05) examined the brain of the toad and came to the conclusion that the hind part of the brain affects metamorphosis, since, when it is removed, the processes are arrested. He lays stress on the fact that the removal of the brain effects the changes described only in case the animal is operated upon before the first pair of limbs have grown. Babák ('13) believes that here we have the influence of hypophysis by means of chemical stimuli. Guder-

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natsch ('12, '14) by feeding tadpoles on hypophysis did not bring about—it is true—any changes in the rate of development, but there is no doubt that feeding experiments with a certain organ may be considered as decisive only if their results are positive, a fact which is not well understood by certain investigators. In fact, Adler ('14), Smith ('16), and Allen ('16, '17) confirm that the processes of metamorphosis in tadpoles are arrested by removal of the hypophysis, which, however, is accompanied by changes in the thyroid. On the other hand, Rogers ('18) and Larson ('19) described changes of the hypophysis brought about by removal of the thyroid in tadpoles, and Allen ('18) confirmed the fact that tadpoles deprived of their thyroid do not undergo metamorphosis. The question arises, therefore, whether the essential and decisive factor which regulates the transformation in those animals is the function of the hypophysis or of the thyroid. Hirschler ('18–'19) ascertained that metamorphosis is accelerated by injecting axolotls with iodoform or iodin. From the recent researches made by Swingle ('19*a* and *b*), who succeeded in transforming tadpoles deprived of their thyroid by feeding them on iodin with flour, as well as from his own analogous experiments, Allen ('19) draws the general conclusion that the metamorphosis of amphibians depends on iodin stored by the thyroid, and that its secretory function is regulated by the hypophysis.

The relationship of the ganglia of the head to metamorphosis in insects has been studied by Conte and Vaney ('11). These authors made ligatures between the head and the body of caterpillars of *Lymantria dispar* L. and obtained adult moths. They infer from their experiments that the ganglia of the head are of no importance for these processes. The following pages show, however, that their conclusion is false. We shall see that the brain (ganglion supra-œsophageale) exerts a great influence on metamorphosis of insects, and the cause of the erroneous conclusion will be clearly explained.

If we wish to investigate the relation which probably exists between the nervous system and the processes of metamorphosis of insects, we must first of all decide whether, after the removal of single parts of the nervous system, the rate of metamorphosis is in general the same as in normal animals, whether it undergoes ac-

celeration or retardation, or whether it is completely arrested. The problem was to find out a possibly well-defined center which would have a more or less decisive influence on the time of inception or on the rate of metamorphosis. Further, we must consider the means by which this hypothetical influence could act on the processes of metamorphosis. The mechanism of this influence ought to be studied by means of special experiments, and the time at which the influence begins to act ought to be found out. In order to answer this question, I operated on female caterpillars of *Lymantria dispar* L. after their last moult. Thus I was able to consider only the processes of the pupation of caterpillars and the emergence of the adult moths, without taking into consideration the processes of the larval moult.

It must be distinctly remarked that the whole material, experimental as well as that used as a control, came from eggs of one female only. All the caterpillars were reared in precisely identical conditions. Moulting, pupation, and emergence of moths were controlled every 12 hours; thus the error in determining the length of life of caterpillars and chrysalids (see Tables I. and II.) could not exceed 24 hours.

As to the methods of removal of single ganglia, cf. operations in my former paper (Kopeč, '18).

A. The Influence of the Brain on the Inception of the Processes of Metamorphosis.

The behavior of caterpillars of *Lymantria dispar* L. deprived of their brain (ganglion supra-œsophageale) allows us to draw the conclusion that this ganglion has a quite specific quality, very important to the whole organism, and characterizing the brain as an organ which excites histolytical processes in the caterpillar and regulates the time of inception of the general processes of pupation. Let us consider the data given in Table I., which refer to the pupation or to the death of caterpillars, whether normal, brainless, or those used as a control, viz., specimens which have been injured in the same manner as the caterpillars deprived of their brain, and which, in spite of the presence of the brain, refused food as well as the brainless specimens. All these specimens (exclu-

sively females) were operated upon on the seventh day after their last moult and lived under the same conditions of heat, light, and humidity. We see that out of 25 brainless caterpillars only 5 underwent pupation, and in these the instant of beginning pupation exhibits a certain retardation in comparison with the behavior of the specimens used as a control. It is evident that the majority of the brainless caterpillars did not undergo metamorphosis, remained alive for weeks, and did not die until the whole store of fat in their body had been completely exhausted. Large full-blooded and fat caterpillars of 4–5 cm. in length become small during this period, and were often scarcely 1 cm. long in their final state. At the same time their bodies shrank and almost completely dried up, their movements became fainter and fainter, until at last they died. Caterpillars deprived of their brain do not, as a rule, die after the operation as a result of any disease induced by the removal of the brain, but, on the contrary, they live relatively very long. Only a few specimens die soon after operation as a consequence of mechanical injuries taking place during the removal of the brain. The essential difference between these insects and those used as a control lies only in the fact that histolytical processes subsequently followed by metamorphosis can begin only in animals the brain of which has exerted a proper stimulus at the proper time. It is certain that the few brainless caterpillars which succeeded in becoming pupæ attained the stage only because the brain had already influenced the tissues before its removal. In this function, too, the animals exhibit individual fluctuations; here we evidently see specimens in which the brain had begun to perform its stimulative part very early, or had exerted it very quickly. It ought to be admitted that, if we could succeed in feeding brainless specimens, their life could be still longer. These caterpillars, however, would probably not undergo metamorphosis.

The opinion that the brain is of importance for metamorphosis is supported by the following observations, which prove also that the influence discussed sets in at a somewhat definite period, viz., in females of *Lymantria dispar* L. in my breed between the seventh and tenth day after their last moult. Let us observe the behavior of the caterpillars which were deprived of their brain not on the seventh day after the last moult as in investigations hitherto con-

TABLE I. BEHAVIOR OF THE FEMALE CATERPILLARS OPERATED UPON ON THE SEVENTH DAY AFTER THEIR LAST MOULT.
p. = pupation; d. = death of caterpillars.

Caterpillars Deprived of the Brain.			Caterpillars Deprived of the Subœsophageal Ganglion (Brain Intact).			Caterpillars Used as a Control; Injuries of the Head as in Caterpillars Deprived of Brain (Brain Intact).			Normal Caterpillars.		
Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.
1	After 12½ days p.	—	1	After 7½ days p.	—	1	After 6½ days p.	21	1	After 6½ days p.	19
2	" 12½ " p.	19	2	" 8½ " p.	17	2	" 6½ " p.	21	2	" 7 " p.	20½
3	" 14 " d.		3	" 8½ " p.	17	3	" 7 " d.		3	" 7½ " p.	20
4	" 14½ " p.	17	4	" 9 " p.	20½	4	" 7½ " p.	21	4	" 8½ " p.	20
5	" 14½ " p.	—	5	" 9½ " p.	18½	5	" 7½ " p.	—	5	" 8½ " p.	18½
6	" 15 " d.		6	" 9½ " p.	20	6	" 8 " p.	22	6	" 8½ " p.	22
7	" 15 " d.		7	" 10 " d.		7	" 8 " p.	20½	7	" 8½ " p.	20
8	" 15 " d.		8	" 10 " p.	19½	8	" 8 " p.	18½	8	" 8½ " p.	18
9	" 16 " d.		9	" 12½ " p.	16	9	" 8½ " p.	22	9	" 9 " p.	20½
10	" 16 " d.		10	" 12½ " p.	19½	10	" 8½ " p.	—	10	" 9 " p.	17
11	" 16½ " p.	21	11	" 12½ " p.	18	11	" 9 " p.	21½	11	" 9 " p.	18
12	" 17 " d.		12	" 14 " p.	17	12	" 9 " p.	22½	12	" 9½ " p.	18
13	" 17½ " d.		13	" 14 " p.	19	13	" 10½ " p.	21	13	" 9½ " p.	17
14	" 17½ " d.					14	" 11 " d.		14	" 9½ " p.	17
15	" 21½ " d.					15	" 11½ " d.		15	" 10 " p.	20½
16	" 22½ " d.					16	" 12 " p.	17	16	" 10 " p.	19
17	" 22½ " d.								17	" 10 " p.	21½
18	" 23 " d.								18	" 13 " p.	19
19	" 23 " d.										
20	" 23 " d.										
21	" 25 " d.										
22	" 25 " d.										
23	" 27 " d.										
24	" 31 " d.										
25	" 31 " d.										

sidered, but three days later. From Table II. it is evident that here the percentages are quite different: almost all caterpillars undergo pupation. The specimens which did not succeed in becoming pupæ live no longer than the normal larvæ and their death evidently results from the injuries of the operation. In this case the metamorphosis of the caterpillars operated upon does not undergo any retardation.

The above-mentioned experiments of Conte and Vaney ('11) must have been performed on such older specimens. In my previous paper (Kopeć, '12), in which, like the authors mentioned, I stated that the nervous system is unimportant up to the time of pupation, I relied only on full-grown specimens; in view of my present data, the former opinion can no longer be upheld. It follows from further operations that none of the other nervous ganglia (in contrast to the brain) has any influence on the time of pupation.

The dependence of the metamorphosis of the insect on its brain, already discussed, and its independence of the other ganglia, may be observed in one and the same specimen by means of a special method of cutting the caterpillars. Having made ligatures with strong silk round the body of the caterpillar into places chosen *ad libitum,* and having made a section between the ligatures, we may obtain two separate parts from one caterpillar, both of which are able to live. For instance, in one series of experiments the caterpillars were operated upon 7 days after their last moult, and it was found that the parts composed of the head and a few segments of the larval body attained the stage of pupation in from 7 to 9 days after the time of operation. Larger or smaller parts of the hind segments of the body devoid of the brain did not succumb till 35 days after the operation, but at death they exhibited no traces of histolytical processes. When caterpillars a few days older were operated upon both fragments underwent pupation at the normal time.

B. The Nature of the Influence Exerted by the Brain on the Course of Metamorphosis.

If the influence exerted by the brain on the course of metamorphosis were by means of nerves, it might be supposed that the

TABLE II. BEHAVIOR OF THE FEMALE CATERPILLARS OPERATED UPON ON THE TENTH DAY AFTER THEIR LAST MOULT.

p. = pupation; d. = death of caterpillars.

Caterpillars Deprived of the Brain.			Caterpillars Deprived of the Subœsophageal Ganglion (Brain Intact).			Caterpillars Used as a Control; Injuries of the Head as in Caterpillars Deprived of Brain (Brain Intact).			Normal Caterpillars		
Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.
1	After 3 days p.	—	1	After $3\frac{1}{2}$ days p.	21	1	After 4 days p.	$19\frac{1}{2}$	1	After 3 days p.	20
2	" $3\frac{1}{2}$ " p.	—	2	" $3\frac{1}{2}$ " p.	—	2	" 4 " p.	$20\frac{1}{2}$	2	" 4 " p.	21
3	" 4 " p.	21	3	" $4\frac{1}{2}$ " p.	—	3	" 5 " p.	$19\frac{1}{2}$	3	" 5 " p.	20
4	" 4 " p.	22	4	" 5 " p.	$19\frac{1}{2}$	4	" 5 " p.	$19\frac{1}{2}$	4	" 5 " p.	$20\frac{1}{2}$
5	" 4 " p.	22	5	" 5 " p.	$18\frac{1}{2}$	5	" $5\frac{1}{2}$ " p.	$17\frac{1}{2}$	5	" 5 " p.	21
6	" 4 " p.	21	6	" 5 " p.	$18\frac{1}{2}$	6	" 6 " p.	—	6	" $5\frac{1}{2}$ " p.	$20\frac{1}{2}$
7	" 4 " p.	21	7	" 7 " p.	$17\frac{1}{2}$	7	" $6\frac{1}{2}$ " p.	18	7	" 6 " p.	20
8	" $4\frac{1}{2}$ " p.	—	8	" 7 " d.		8	" $8\frac{1}{2}$ " p.	—	8	" $6\frac{1}{2}$ " p.	$20\frac{1}{2}$
9	" $5\frac{1}{2}$ " p.	21				9	" 9 " p.	17	9	" 8 " p.	19
10	" 7 " d.					10	" $10\frac{1}{2}$ " d.		10	" $8\frac{1}{2}$ " p.	$18\frac{1}{2}$
11	" 8 " d.										
12	" $8\frac{1}{2}$ " p.	—									
13	" $9\frac{1}{2}$ " d.										
14	" 10 " p.	$17\frac{1}{2}$									
15	" $10\frac{1}{2}$ " p.	19									

removal of the subœsophageal ganglion would have the same effect as the removal of the brain, as in this case the continuity between the brain and the remainder of the central nervous system is disturbed. But the corresponding data of Tables I. and II. prove that in this case the insects behave normally. The behavior of insects which have been deprived of two or three successive ganglia of the thorax or of the abdomen was also similar: both parts of the body, the part situated in front of the place of operation and that behind it, attained the stage of pupation simultaneously, although the connection between the central nervous system of the two parts had been quite destroyed. After several trials, I convinced myself that processes of regeneration never occur, such as would be able to produce a new nervous communication between the two parts.

At most it must be admitted that the brain is connected with the part of the nervous system situated behind the place of operation by means of the "intestinal nervous system," which is composed of very small ganglia lying in the tissue surrounding the œsophagus and connected in front with the brain. (The "sympathetic nervous system" of the caterpillar undergoes rupture during the removal of the ganglia of the nervous chain. For corresponding anatomical data see the paper of Cattie, '80.) Owing to the unusually small size of the intestinal system, it was impossible to study it in greater detail by operating. On the other hand, I do not consider it possible that any nervous conduction between the two parts of the larval body operated upon can take place, since I observed several times that the part of the body behind the segment deprived of its nervous ganglion does not respond to any stimuli exerted on the fore-part of the caterpillar operated upon, and *vice versa.* The fore-segments of the operated body may be sharply pinched, injured, cut, or burned, still the hind part does not perform even the slightest movements referable to the stimuli, although under normal conditions even slight excitations of any part of the body produce violent reflexes. In regard to this, there seems to be no doubt that the fore-part of the body of the operated caterpillars is in no nervous connection with the part situated beyond the place of operation. It is, therefore, most probable that the brain does not influence the general processes of metamorphosis

through the nerves, but that it has rather the function of an organ of internal secretion, in that it affects the organism by means of a substance (or substances) which may be supposed to pass into the blood of the caterpillar from the brain at a certain stage of the larval life. Experiments bearing on the transplantation either of the organized brain or of its matter might perhaps decide whether this conclusion is true. It is most probable that the chemical substance (or substances) here acting is the ferment called "thyrosinase," the aggregation of which during the pupation of insects and the disappearance of which at the end of the pupal life has been previously noticed by Dewitz ('05, '16) and more recently by Steche and Waentig ('13). In his numerous papers Dewitz long ago ascribed an important "rôle" to "thyrosinase" in metamorphotic processes.

The mere observation that the brain has an influence on the inception of histolytical processes in the body of caterpillars gives no idea as to the physiological nature of this influence. The method of removal of the brain from the larval body was not adapted to ascertain whether the proper stimulus effected by the brain is the only factor which excites histolytical processes in the body of the caterpillar, or whether certain physiological changes which appear in the organism coöperate spontaneously and independently of the brain in the same direction. In other words, does the larval organism undergo metamorphosis exclusively because of the stimulus derived from the brain, or is the brain able to act only when certain physiological changes occur in the tissues of the caterpillar, changes without which the influence of the brain must remain powerless? The investigation in this direction had to be made by transplanting the organs of young caterpillars in which these hypothetical physiological changes in the tissues themselves had not yet occurred onto full-grown caterpillars, in which the influence of the brain on the excitement of metamorphosis might already be noticed. Should the grafted organs exhibit acceleration in their development as a consequence of their new surroundings, the metamorphosis of the insects would have to be considered as dependent primarily on the brain, the latter having caused the transformation of the larval organs independently of their age or physiological development. Should this hypothesis prove false,

the coöperation of stimulus of the brain and of the physiological conditions of larval tissues (without which the influence of the brain would have no effect) would appear to be indispensable.

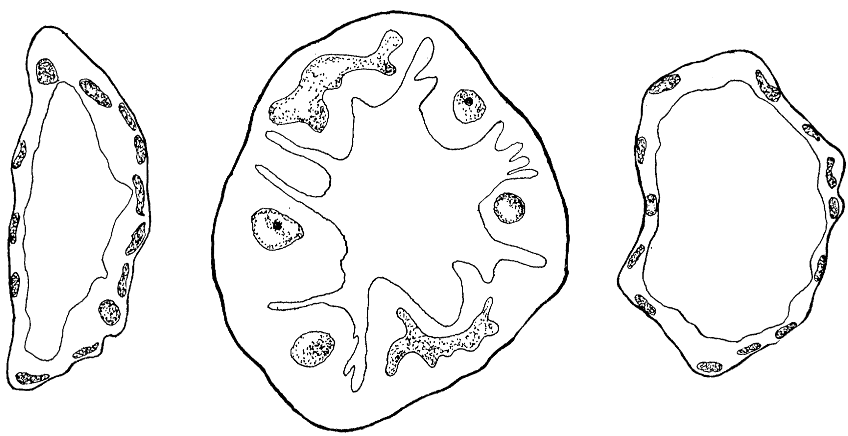
For these transplantations I used the sexual glands. The gonads of moths do not belong to those organs which develop in the chrysalis from the special imaginal discs; they are formed by the growth and histological changes of the small larval sexual glands whose existence has been observed for a long time in caterpillars. All four ovarial tubes of caterpillars hitherto contained in a connective tissue membrane and forming together with it one apparently globular body grow during the caterpillar's pupation to such a degree that they break out of the membrane. In the testes, on the other hand, the characteristic high cylindrical epithelium which lines the interior of that part of the developing spermiduct adjacent to the testicles is formed at that period from the uniform mass of imperfectly separated cells situated at the base of the gland. At the same time the testicle shrinks considerably and its contents become much compacter. I mention these facts in somewhat greater detail than usual in order to emphasize that, although they do not develop from imaginal discs, the sexual glands exhibit certain distinct evolutive changes during their development. As a consequence of this the study of the behavior of the organs after the transplantation is quite suitable for our present purpose. The ovaries and testicles of caterpillars after the third or fourth moult were transferred to full-grown caterpillars which were to undergo pupation in a few days. Hence, in these caterpillars, the substance which excites histolytical processes had already passed over from the brain into the body. It was found that the rate of evolution of the testes and the ovaries grafted remained unchanged, notwithstanding the new surroundings in which the processes of metamorphosis were just beginning or had already begun. From this follows the important conclusion that the stimulus of the brain is inadequate for metamorphosis of the separate organs of insects, and that it is only able to act when the organism, having attained a certain stage of development, is prepared physiologically to respond to the stimulus.

C. The Independence of the Further Processes of Metamorphosis.

I have already mentioned that the brain exerts an influence on the inception of processes of metamorphosis at a certain definite period of the larval life, in females of *Lymantria dispar* L. of my breed between the seventh and tenth day after their last moult. The question arises as to whether this influence, which excites the inception of histolytical processes in caterpillars, at the same time makes the further course of metamorphosis possible, leading to the formation of the imago; or whether brainless chrysalids are unable to become moths. When the caterpillar has been influenced by the brain sufficiently to undergo pupation, the further evolutive processes, which take place in chrysalids, have been shown to occur independently of the brain. By comparing the data relative to the length of life of the chrysalids in various specimens (brainless, those used as standard, and normal) we see, moreover, that even the rate of formation of the imago from the chrysalis undergoes neither retardation nor acceleration when the brain is removed (cf. Tables I. and II.). If a caterpillar deprived of its brain undergoes pupation, the emergence of the moth from the chrysalis takes place at the normal time. The removal of the brain or of other ganglia has merely a local effect in that (as I want to point out in another paper) muscles are completely or almost completely absent in the corresponding segments. The absence of muscles, however, is the result of merely local correlation between the presence of the imaginal muscles and that of the nervous ganglion in a segment of the body, but it has no connection with the influence under discussion—*i.e.,* the influence of the larval brain on the *whole* histolytical and evolutive processes during the metamorphosis of insects in all tissues of the *whole* body.

The following experiments were made to demonstrate the above principle, viz., that the further metamorphosis of insects, having once been excited by the influence of the brain, continues independently in a different manner. Evidence ought to be furnished by the metamorphosis of imaginal discs of single parts of the insectal body, if it should take place independently of the surroundings to which they had been artificially transferred. According to

recent researches on the development of the Malpighian tubes in moths, the cells of the tubes of full-grown insects are derived directly from the cells of the larval tubes through certain physiological and morphological metamorphosis. (Cf. paper of Samson, '08.) By grafting fragments of the larval Malpighian tubes into the head or thorax, I hoped to solve the problem as to whether the metamorphosis of these tubes might occur normally, in anomalous surroundings, even in the absence of a connection between the tubes and the intestine. Smaller or larger parts of the tubes taken



A *B* *C*

from various regions were grafted into the head or the thorax of the same caterpillar after the last moult of the animal: some of the caterpillars operated upon had just accomplished this moult, others had lived already 10 or 11 days after the last moult. In other cases the Malpighian tubes were transplanted from female caterpillars 10 days after their last moult into other caterpillars 2 or 3 days after this moult. After a few days the ends of these implanted parts were healed up, and so the contents could no longer come out. In the adult insect or pupa I several times succeeded in detecting microscopically the presence of the implanted tubes, which were here not united with the intestine. The cells of the tube which developed in the head or the thorax (cf. Fig. *A* in text) exhibited different characters from those observed in the cater-

pillars (Fig. *B*), viz., they corresponded to the normal cells of tubes in the normal adult moth (Fig. *C*). Their height was far lower than in the larval stage of development, sometimes they were twice as low. Their nuclei, having a much more compact chromatine, and a flatter, non-ramified form, were situated nearer the base of the cells. The interior margin of the tubes was not so much notched as in the caterpillar. All these characters prove that the cells of the implanted tubes have undergone normal metamorphosis. This metamorphosis of the Malpighian tubes is consequently independent of the function of this organ, as it is difficult to suppose that the tubes undergoing metamorphosis in heterogeneous surroundings were able to realize their special function in the chrysalis and later in the adult moth, as they were unconnected with the developing intestine, completely grown together and always filled with excrements previously formed during the larval life. If it could be proved without any objection that the Malpighian tubes which have been implanted into the head or thorax had no nervous junction in the new surroundings, the metamorphosis of transplanted fragments of Malpighian tubes would be a further proof for the assertion made in the previous chapter that the brain exerts its influence by means of internal secretion. The probably unchanged rate of metamorphosis of the tubes transferred from caterpillars 10 days after their last moult into those 2 to 3 days after this moult (thus from specimens in which the influence of the brain was already observable into specimens in which there was yet no such influence) points to the conclusion that tissues once stimulated by the brain to transformation undergo further metamorphosis independently.

Should this principle be of general importance, it would be expected that the organs of much older caterpillars (a few days before pupation) grafted on the much younger caterpillars after the fourth moult (and therefore some two to three weeks younger) might also undergo further metamorphosis at the normal rate, in spite of the fact that the organs of the younger specimens do not as yet indicate the slightest trace of the histolytical processes. Transplantations of the germ of wings fully confirmed this hypothesis. I removed the germ of the first left wing from numerous caterpillars shortly before their last moult, and in its place I

grafted an analogous germ derived from a full-grown caterpillar, which was to undergo metamorphosis in a few days. In two of the caterpillars somewhat large and black outgrowths—dwarfed and deformed wings—appeared on the place of grafting. In their uniformly dark hue and the thickness and markings of the chitin they formed a striking contrast to the bright-colored tegument of the caterpillar. (Cf. Fig. *D*.) The small size and the abnormal

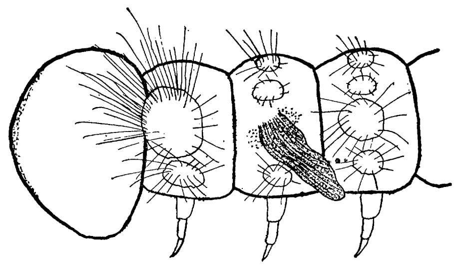


FIG. *D*.

forms were doubtless caused by inevitable injuries of the delicate germ during transplantation, as well as by the difficulty of extracting the pupal wing from the moulting but not pupating caterpillar. Histologically the development of the pupal wings was normal. I have also formerly observed (Kopeć, '11) that the sexual glands of full-grown caterpillars develop at their normal rate when grafted on the caterpillar after their third or fourth moult. Such a behavior of the grafted wings and sexual glands strikingly confirmed the above-stated principle that after the time when the brain has already exerted its influence on the whole organism—which influence decides the inception of histolytical processes leading to the chrysalis stage—the subsequent development and metamorphosis of organs is quite independent of the brain.

The results of my experiments proved that the rate of development of the grafted organs underwent no change due to the influence of new surroundings, differing both as to age and physiological state. These results seem to disagree with analogous researches of Uhlenhuth ('12, '13) on amphibians. This author grafted eyes of salamander larvæ on specimens of a different age and came to the conclusion that the metamorphosis of the transplanted eyes underwent a distinct retardation when grafted on

younger specimens, and an acceleration in the contrary case—*i.e.*, the transplanted as well as the own eyes of the foster-mother underwent metamorphosis at the same time. But the disagreement of these results with the behavior of the transplanted insect organs in my investigations is only apparent. The influence of the surroundings on the grafted eye, observed by Uhlenhuth, was to be remarked only when the age of the specimens on which the eyes were implanted did not differ much from that of animals whose eyes were taken for the operation. In the remaining cases—*i.e.*, under the conditions which alone corresponded to my investigations on the transplantation of organs from young caterpillars after their third or fourth moult to full-grown caterpillars or chrysalids and *vice versa*—the grafted eyes retained their proper developmental rate without being influenced by the new surroundings, just like the organs of insects transplanted in my experiments. Nor do my experiments on insects in any way contradict the experiments of Weigl ('13) which were made simultaneously with but independently of those of Uhlenhuth. Weigl studied the same problem in his investigations on the homo- and heteroplastic transplantation of the skin in salamanders and other amphibians. In regard to the course of the metamorphosis of the grafted pieces of skin, he comes to the same general conclusion as Uhlenhuth. In the experiments of Kornfeld ('14) we can also find a parallel to the results I observed on insects. This author, who transplanted the gill of salamanders, agrees in his final conclusion with Uhlenhuth. Uhlenhuth ('13), mentioning my former experiments on the transplantation of gonads in moths, imputes to me results completely different from those which I really obtained (Kopeć, '11). More recently this error has been corrected by Kornfeld in agreement with Uhlenhuth.

In specimens deprived of the brain or of other nervous ganglia we often may observe certain mechanical difficulties during pupation and emergence of the moth. They are produced by concrescence of the chitin at the place of operation, and cause the appearance of certain deformities of chrysalids or of moths. It must be distinctly noted that all these deformities, caused only by mechanical obstructions during pupation or emergence of the moths, are in no wise dependent on the operation itself, nor conse-

quently on the removal of separate parts of the nervous system. A proof of this is that they also appear—and in the same number of cases—in the specimens with intact nervous system used as a standard and injured similarly as in the operations on the ganglia. Therefore I consider it superfluous to describe these anomalies here.

D. General Conclusions and Summary.

From the whole of this paper it follows that my results relative to the influence of the nervous system on metamorphosis in insects show a very great resemblance to the relations observed in amphibians. According to recent investigations (above mentioned), iodin stored by the thyroid glands in amphibians is the direct cause which elicits metamorphosis, but the production of this substance probably depends on the function of the hypophysis. Consequently in both classes of animals metamorphosis would be controlled by the brain, or part of it. In recent years experiments have been performed on the influence of food containing thyroid or hypophysis on the development of the larvæ of insects, but the results of these researches do not as yet furnish an adequate material for any conclusions. Abderhalden ('19) observed that a part of the moths obtained from caterpillars fed on hypophysis was very large; many specimens from caterpillars fed on thyroid, on the contrary, were very small. These data refer, however, to phenomena of growth, and are possibly quite unrelated to the problem of the rate of transformation here now discussed. Romeis and v. Dobkiewicz ('20) studied the rate of development in larvæ of *Calliphora* fed on thyroid and obtained no distinct changes which might have been attributed exclusively to the specific effect of the thyroid gland. As I mentioned above, negative results of feeding experiments with hypothetical specific influence ought not to be considered as decisive, because it may be inferred from them only that the substance used does not exert any effect on the organism when given *per os*. Hankó ('12), on the other hand, ascertained a very considerable influence of hypophysis on rate of moults in *Asellus aquaticus*. But, as moults in Crustacea are not to be totally identified with moults in insects having complete metamorphosis, these results also have no great importance for our

problem. The whole question demands further research in order to elucidate whether and in what degree there exists an analogy between the physiology of transformation of amphibians and that of insects.

The following summary contains the more important results of this paper:

1. The brain (ganglion supra-œsophageale) of the caterpillar of *Lymantria dispar* L. has particular importance in the general processes of metamorphosis. The presence of the brain is indispensable, at least up to a certain period, for the inception of histolytical processes. The influence of the brain in this direction is probably chemical; hence the brain ought to be considered as a gland of internal secretion.

2. At some well-defined time before pupation the quantity of the corresponding substance (or substances) secreted by the brain is already sufficient for the complete pupation of the caterpillar.

3. Tissues of the caterpillar influenced by the brain undergo further metamorphosis independently; the germs of the wings of caterpillars grafted shortly before pupation on younger caterpillars near their last moult are transformed in the larval organism into pupal wings in spite of the absence of histolytical processes in the new surroundings. Analogical results have been obtained by transplantation of Malpighian tubes and gonads.

4. It follows from other experiments on the transplantation of organs that the stimulus coming from the brain is not sufficient by itself for the metamorphosis of caterpillars: this stimulus acts only when the insect organism, having attained a certain stage, is physiologically prepared to answer to this influence. This is shown by the fact that germs of gonads from young caterpillars grafted into full-grown caterpillars do not undergo metamorphosis characteristic of the pupal development of gonads; in other words, the rate of development underwent no acceleration in spite of the histolytical processes occurring in the new surroundings.

5. Other parts of the nervous system have no influence on the general processes of metamorphosis.

6. By making ligatures round the body of the caterpillar and cutting it in different places, we may obtain fragments consisting of front, middle, or hind segments. The front fragments undergo

metamorphosis in a normal manner, provided the caterpillar does not die of starvation. On the contrary, the middle and hind fragments undergo pupation only if they have belonged to caterpillars which would have pupated in a few days.

7. The Malpighian tubes are transformed into tubes of the adult insect independently of the intestine and of the performance of their specific function.

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